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Supplemental Information

Mediator recruits the cohesin loader Scc2 to RNA Pol II-transcribed genes and promotes sister chromatid cohesion

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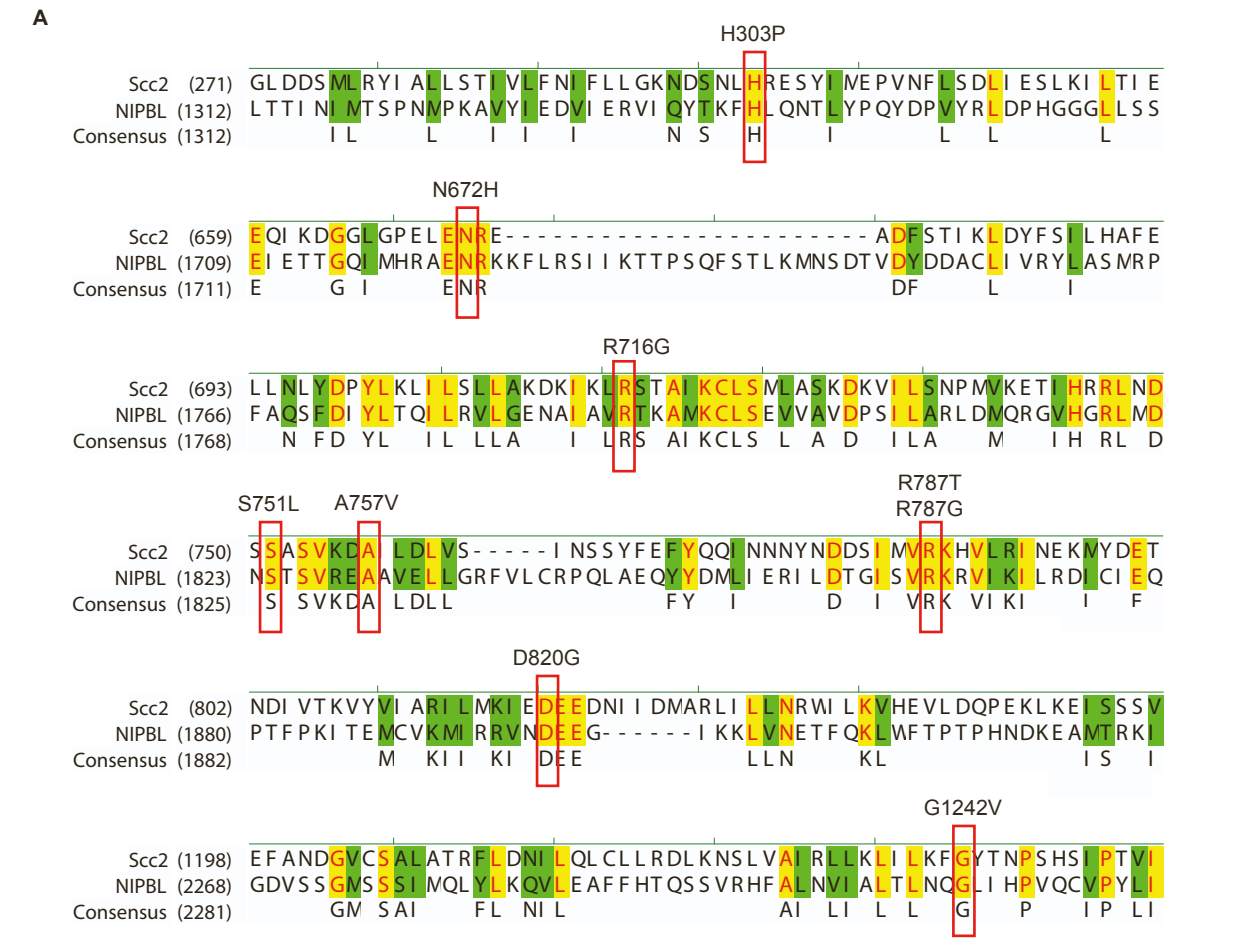
A
H303P
N672H
R716G
S751L
A757V
R787T
R787G
D820G
G1242V
Scc2 (271)
NIPBL (1312)
Consensus (1312)
Scc2 (659)
NIPBL (1709)
Consensus (1711)
Scc2 (693)
NIPBL (1766)
Consensus (1768)
Scc2 (750)
NIPBL (1823)
Consensus (1825)
Scc2 (802)
NIPBL (1880)
Consensus (1882)
Scc2 (1198)
NIPBL (2268)
Consensus (2281)
B
Smc3
Smc1
Scc1
Scc2
Scc2 R787
Smc3
Smc1
Scc1
Scc2
Scc2 G1242

Figure S1. CdLS mutations occurring at amino acids conserved from Nipbl to Scc2, related to Figure 1

(A) Segments of the amino acid alignment of human Nipbl with *Saccharomyces cerevisiae* Scc2. Amino acids that are mutated in CdLS and conserved in Scc2 are boxed in red.
(B) Locations of the R787G and G1242V mutations in the gripping state structure of topological cohesin loading onto DNA. The mutations are indicated in red, R787G falls within a positive patch of amino acid residues that interact with DNA while G1242V is proximal to the interface between Scc2 and the SMC heads.

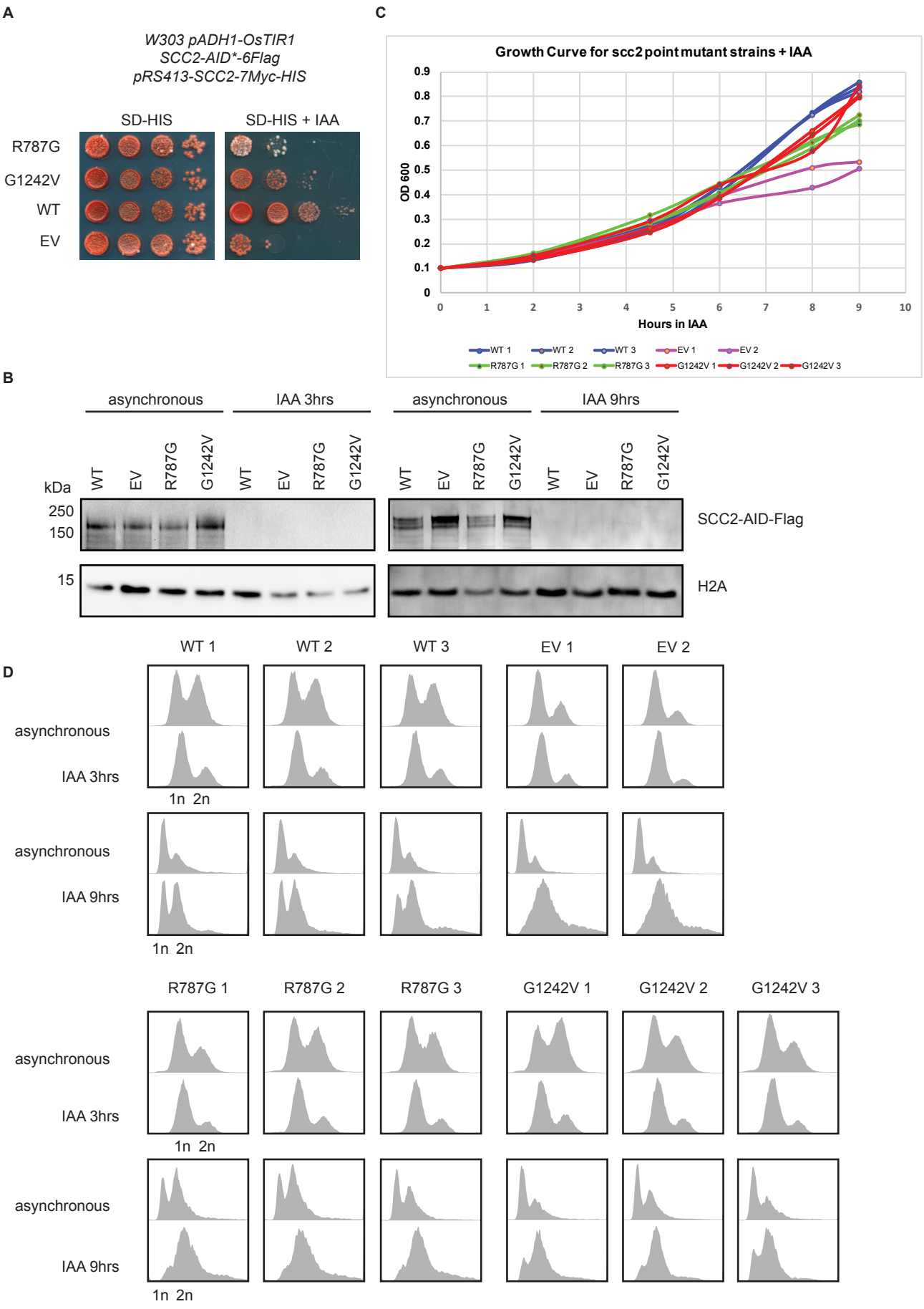
A
W303 pADH1-OsTIR1
SCC2-AID*-6Flag
pRS413-SCC2-7Myc-HIS
SD-HIS
SD-HIS + IAA
R787G
G1242V
WT
EV
C
Growth Curve for scc2 point mutant strains + IAA
OD 600
Hours in IAA
WT 1
WT 2
WT 3
EV 1
EV 2
R787G 1
R787G 2
R787G 3
G1242V 1
G1242V 2
G1242V 3
B
asynchronous
IAA 3hrs
asynchronous
IAA 9hrs
kDa
250
150
15
SCC2-AID-Flag
H2A
D
asynchronous
IAA 3hrs
IAA 9hrs
1n 2n

Figure S2. Validation of Scc2^{degron} and cytometry for Scc2 point mutant RNA-seq experiment, related to Figure 2

(A) 10-fold serial dilutions of Scc2^{degron} strains with the indicated Scc2 mutant expressed from a HIS selectable plasmid were spotted on SD-HIS and SD-HIS+IAA.
(B) Cycling cells were treated with IAA for 3 hours and 9 hours. Cells were pelleted and lysate prepared and analyzed for depletion of Scc2 by immunoblot.
(C) Growth curve of yeast strains used for Scc2 point mutant RNA-seq. Cycling cells were diluted to OD_{600} ~ 0.1 in SD-HIS media at 30° C. IAA was added to a concentration of 1mM and OD_{600} was monitored for 9 hours.
(D) FACS analysis of DNA content of yeast cells used in the RNA-seq experiment shown in Figure 2.

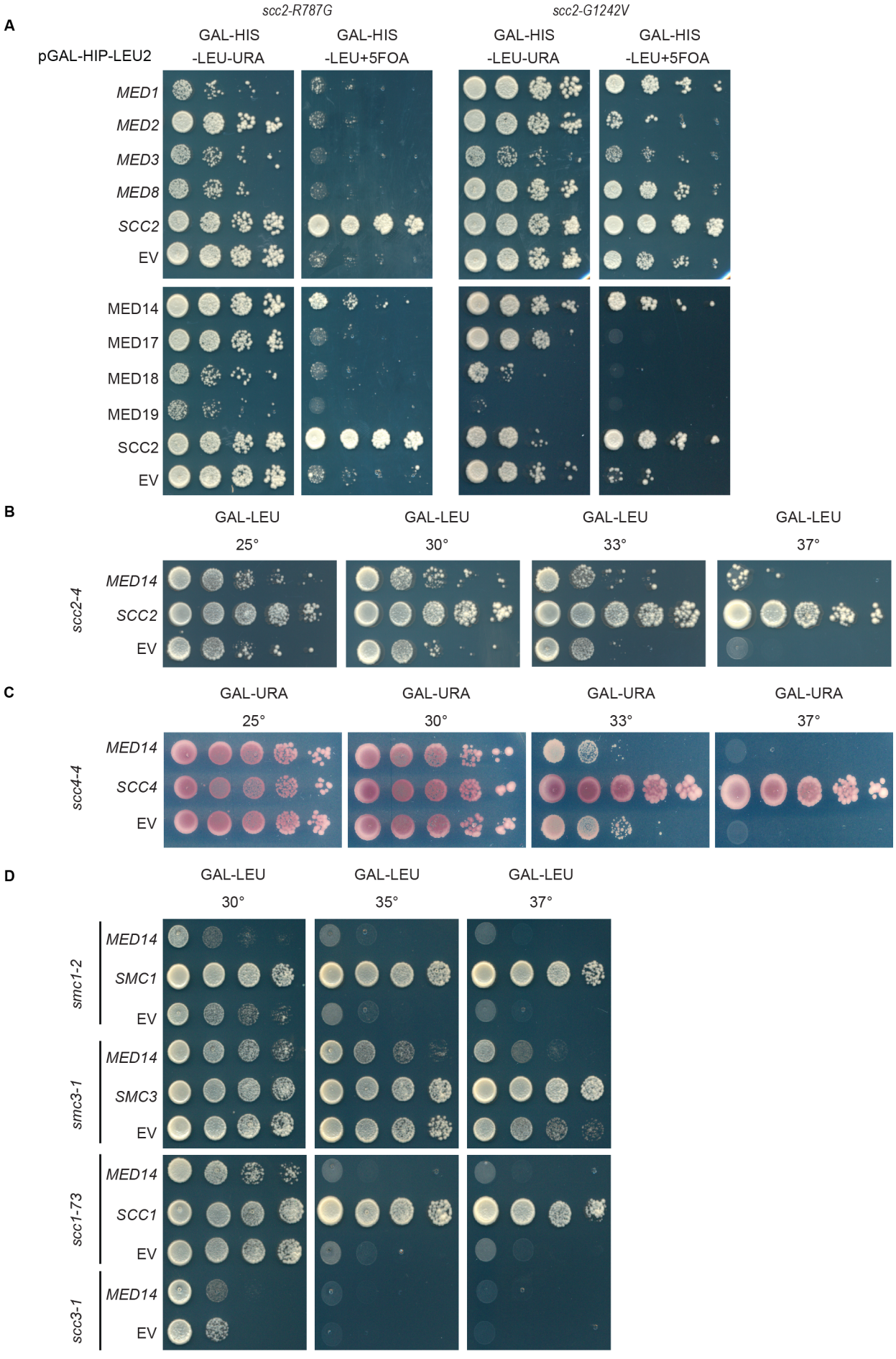
A
scc2-R787G
scc2-G1242V
pGAL-HIP-LEU2
GAL-HIS -LEU-URA
GAL-HIS -LEU+5FOA
GAL-HIS -LEU-URA
GAL-HIS -LEU+5FOA
MED1
MED2
MED3
MED8
SCC2
EV
MED14
MED17
MED18
MED19
SCC2
EV
B
GAL-LEU
25°
30°
33°
37°
scc2-4
MED14
SCC2
EV
C
GAL-URA
25°
30°
33°
37°
scc4-4
MED14
SCC4
EV
D
GAL-LEU
30°
35°
37°
smc1-2
MED14
SMC1
EV
smc3-1
MED14
SMC3
EV
scc1-73
MED14
SCC1
EV
scc3-1
MED14
EV

Figure S3. Growth assays to investigate the genetic interaction of Mediator with Scc2 and cohesin, related to Figure 3

(A) Suppression of *scc2*^{R787G} and *scc2*^{G1242V} is specific to *MED14.* Multiple subunits of Mediator were cloned into a *LEU2* selectable galactose overexpression plasmid and transformed into the *scc2*^{R787G} and *scc2*^{G1242V} plasmid shuffle strains. 10-fold serial dilutions of the indicated strains were spotted on Gal-HIS-LEU-URA and Gal-HIS-LEU+5FOA.
(B) *Med14 is a suppressor of scc2-4.* The *MED14, SCC2,* and empty vector (EV) *LEU2* selectable galactose overexpression plasmids were transformed into the *scc2-4* temperature sensitive strain. 10-fold serial dilutions of the indicated strains were spotted on Gal-LEU and incubated at the indicated temperatures.
(C) *MED14* overexpression does not rescue *scc4-4*. The *MED14, SCC4,* and empty vector (EV) *URA3* selectable galactose overexpression plasmids were transformed into the *scc4-4* temperature sensitive strain. 10-fold serial dilutions of the indicated strains were spotted on Gal-URA and incubated at the indicated temperatures.
(D) *MED14* overexpression does not rescue cohesin mutants. LEU2 selectable galactose overexpression plasmids containing *MED14* and the *SMC1*, *SMC3*, *SCC1*, and *SCC3* subunits of cohesin were transformed into the indicated temperature sensitive cohesin mutant strains. 10-fold serial dilutions of the indicated strains were spotted on Gal-LEU and incubated at the indicated temperatures.

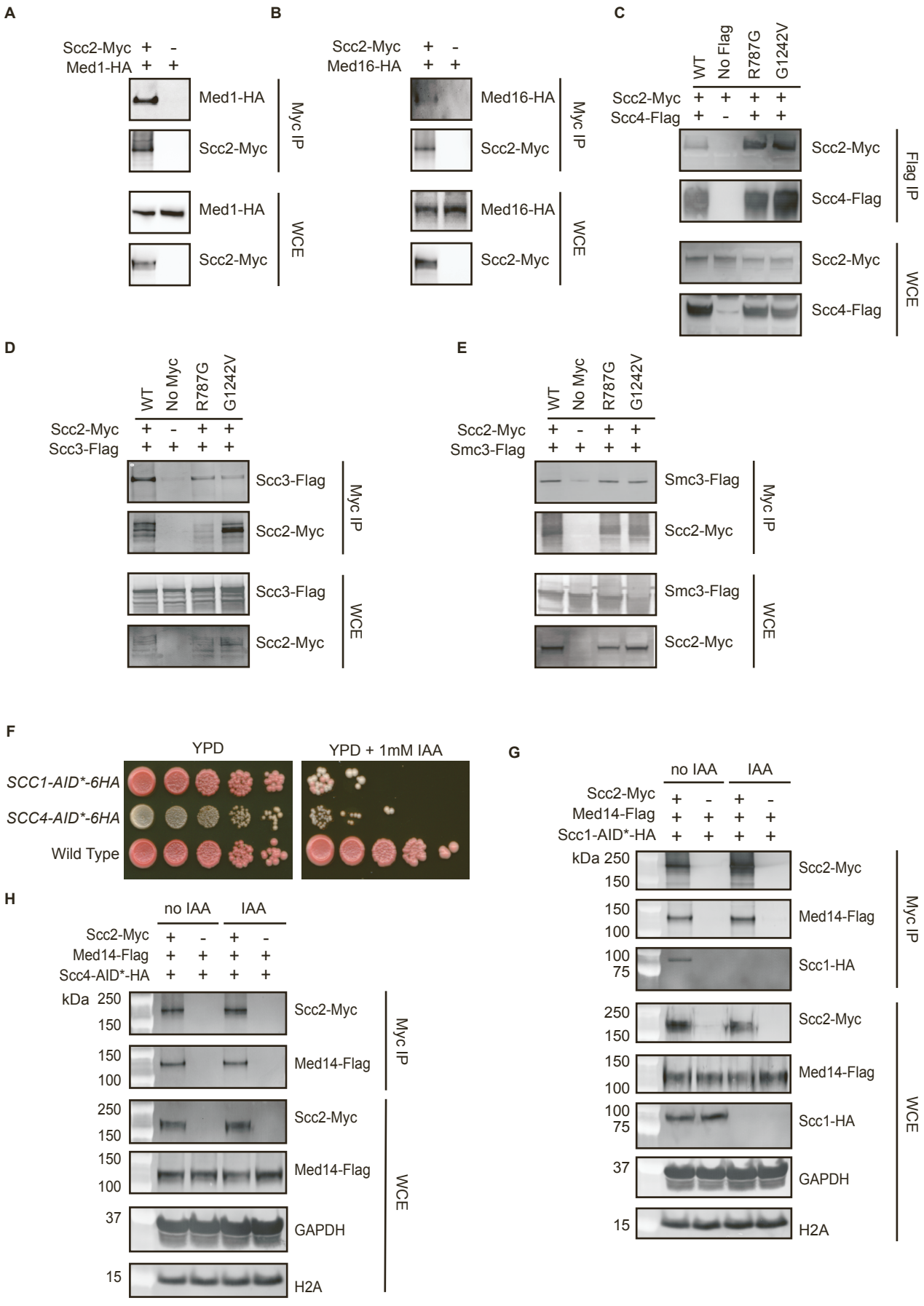
A
Scc2-Myc + -
Med1-HA + +
Med1-HA
Scc2-Myc
Myc IP
Med1-HA
Scc2-Myc
WCE
B
Scc2-Myc + -
Med16-HA + +
Med16-HA
Scc2-Myc
Myc IP
Med16-HA
Scc2-Myc
WCE
C
WT
No Flag
R787G
G1242V
Scc2-Myc + + + +
Scc4-Flag + - + +
Scc2-Myc
Scc4-Flag
Flag IP
Scc2-Myc
Scc4-Flag
WCE
D
WT
No Myc
R787G
G1242V
Scc2-Myc + - + +
Scc3-Flag + + + +
Scc3-Flag
Scc2-Myc
Myc IP
Scc3-Flag
Scc2-Myc
WCE
E
WT
No Myc
R787G
G1242V
Scc2-Myc + - + +
Smc3-Flag + + + +
Smc3-Flag
Scc2-Myc
Myc IP
Smc3-Flag
Scc2-Myc
WCE
F
YPD
YPD + 1mM IAA
SCC1-AID*-6HA
SCC4-AID*-6HA
Wild Type
G
no IAA
IAA
Scc2-Myc + - + -
Med14-Flag + + + +
Scc1-AID*-HA + + + +
kDa 250
150
Scc2-Myc
150
100
Med14-Flag
100
75
Scc1-HA
Myc IP
250
150
Scc2-Myc
150
100
Med14-Flag
100
75
Scc1-HA
37
GAPDH
15
H2A
WCE
H
no IAA
IAA
Scc2-Myc + - + -
Med14-Flag + + + +
Scc4-AID*-HA + + + +
kDa 250
150
Scc2-Myc
150
100
Med14-Flag
Myc IP
250
150
Scc2-Myc
150
100
Med14-Flag
37
GAPDH
15
H2A
WCE

Figure S4. Additional interaction analyses between Scc2 and Mediator, related to Figure 4

(A) Scc2 interaction with Med1. Myc tagged Scc2 was immunoprecipitated from yeast whole cell extract. Co-precipitation of Flag tagged Med1 was analyzed by immunoblotting.
(B) Scc2 interaction with Med16. Myc tagged Scc2 was immunoprecipitated from yeast whole cell extract. Co-precipitation of Flag tagged Med16 was analyzed by immunoblotting.
(C) Mutant Scc2 interaction with Scc4. Flag tagged Scc4 was immunoprecipitated from yeast whole cell extract. Co-precipitation of Myc tagged Scc2 was analyzed by immunoblotting.
(D) Mutant Scc2 interaction with Scc3 subunit of cohesin. Myc tagged Scc2 was immunoprecipitated from yeast whole cell extract. Co-precipitation of Flag tagged Scc3 was analyzed by immunoblotting.
(E) Mutant Scc2 interaction with Smc3 subunit of cohesin. Myc tagged Scc2 was immunoprecipitated from yeast whole cell extract. Co-precipitation of Flag tagged Smc3 was analyzed by immunoblotting. Non-specific binding of Smc3 is present in the No Myc control pulldown but at lower levels than the Scc2-Myc immunoprecipitations.
(F) Validation of $Scc1^{degron}$ and $Scc4^{degron}$ strains. 10-fold serial dilutions were spotted on YPD and YPD+IAA.
(G) Interaction of Scc2 and Mediator is not mediated by cohesin. Cycling cells were treated with auxin to deplete the Scc1 subunit of cohesin. Myc tagged Scc2 was immunoprecipitated from normalized yeast whole cell extracts and co-precipitation of Flag tagged Med14 and was analyzed by immunoblotting. Depletion of Scc1 was confirmed by immunoblotting with HA antibody. GAPDH and H2A served as loading controls.
(H) Interaction of Scc2 and Mediator is not mediated by Scc4. Cycling cells were treated with auxin to deplete the Scc4 subunit of the loading complex. Myc tagged Scc2 was immunoprecipitated from normalized yeast whole cell extracts and co-precipitation of Flag tagged Med14 and was analyzed by immunoblotting. The HA tagged degron was not visible by Western blot in this strain. GAPDH and H2A served as loading controls.

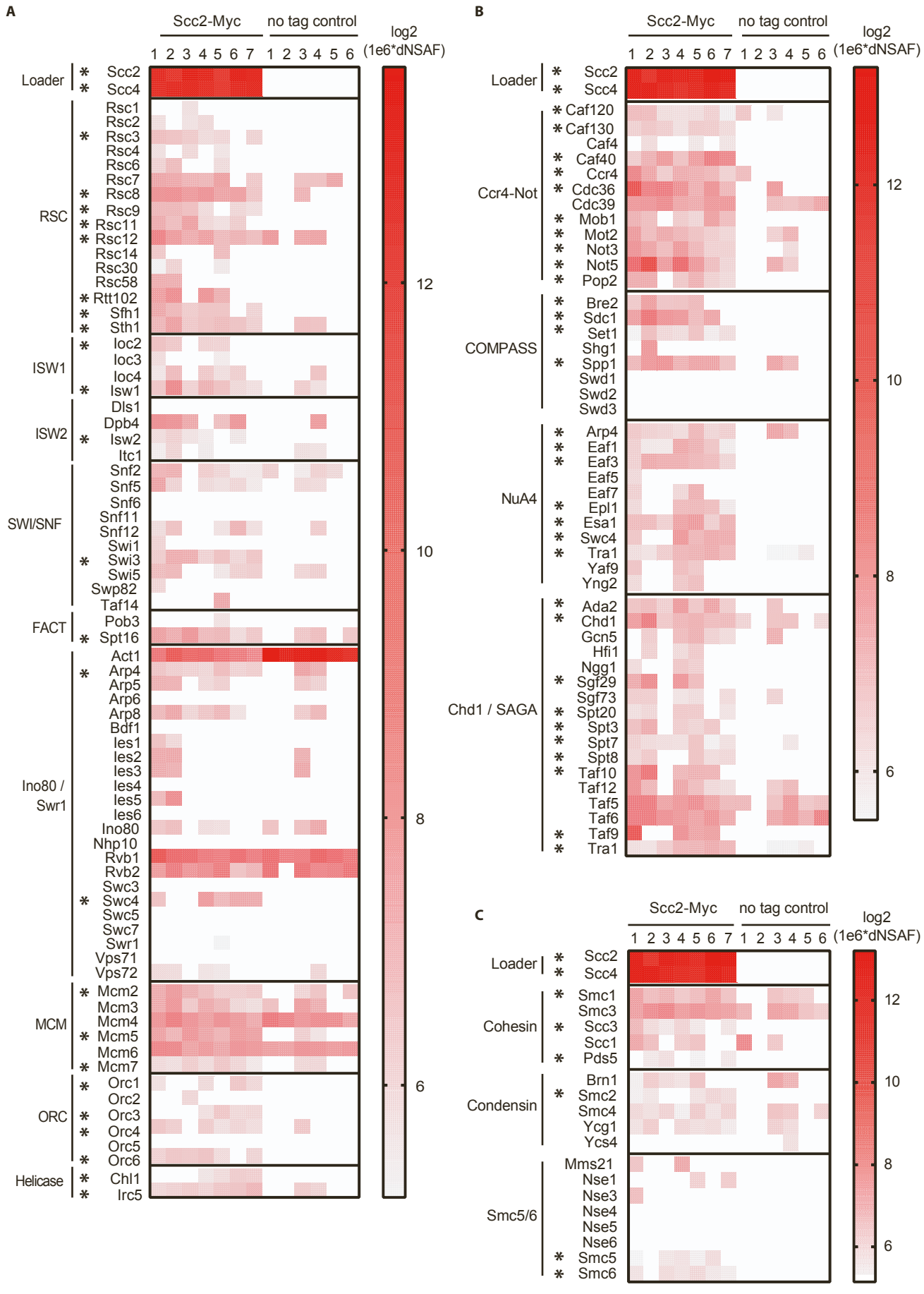
A
Scc2-Myc
no tag control
log2 (1e6*dNSAF)
1 2 3 4 5 6 7 1 2 3 4 5 6
Loader
Scc2
Scc4
RSC
Rsc1
Rsc2
Rsc3
Rsc4
Rsc6
Rsc7
Rsc8
Rsc9
Rsc11
Rsc12
Rsc14
Rsc30
Rsc58
Rtt102
Sfh1
Sth1
ISW1
Ioc2
Ioc3
Ioc4
Isw1
ISW2
Dls1
Dpb4
Isw2
Itc1
SWI/SNF
Snf2
Snf5
Snf6
Snf11
Snf12
Swi1
Swi3
Swi5
Swp82
Taf14
FACT
Pob3
Spt16
Ino80 / Swr1
Act1
Arp4
Arp5
Arp6
Arp8
Bdf1
Ies1
Ies2
Ies3
Ies4
Ies5
Ies6
Ino80
Nhp10
Rvb1
Rvb2
Swc3
Swc4
Swc5
Swc7
Swr1
Vps71
Vps72
MCM
Mcm2
Mcm3
Mcm4
Mcm5
Mcm6
Mcm7
ORC
Orc1
Orc2
Orc3
Orc4
Orc5
Orc6
Helicase
Chl1
Irc5
12
10
8
6
B
Scc2-Myc
no tag control
log2 (1e6*dNSAF)
1 2 3 4 5 6 7 1 2 3 4 5 6
Loader
Scc2
Scc4
Ccr4-Not
Caf120
Caf130
Caf4
Caf40
Ccr4
Cdc36
Cdc39
Mob1
Mot2
Not3
Not5
Pop2
COMPASS
Bre2
Sdc1
Set1
Shg1
Spp1
Swd1
Swd2
Swd3
NuA4
Arp4
Eaf1
Eaf3
Eaf5
Eaf7
Epl1
Esa1
Swc4
Tra1
Yaf9
Yng2
Chd1 / SAGA
Ada2
Chd1
Gcn5
Hfi1
Ngg1
Sgf29
Sgf73
Spt20
Spt3
Spt7
Spt8
Taf10
Taf12
Taf5
Taf6
Taf9
Tra1
12
10
8
6
C
Scc2-Myc
no tag control
log2 (1e6*dNSAF)
1 2 3 4 5 6 7 1 2 3 4 5 6
Loader
Scc2
Scc4
Cohesin
Smc1
Smc3
Scc3
Scc1
Pds5
Condensin
Brn1
Smc2
Smc4
Ycg1
Ycs4
Smc5/6
Mms21
Nse1
Nse3
Nse4
Nse5
Nse6
Smc5
Smc6
12
10
8
6

Figure S5. Scc2 interacts with multiple chromatin remodelers, chromatin modifiers, and all three SMC complexes in yeast, related to Figure 4

Crosslinking mass spectrometry was performed on Myc tagged Scc2 purified from whole cell extract with anti-Myc magnetic beads with on bead DSSO crosslinking and on bead digestion. Heatmaps of 1e6 log2 transformed dNSAF values show proteins enriched in the Scc2 purifications compared to the no tag control. Asterisks indicate statistical significance at $p < 0.05$.
(A) Heatmap of chromatin remodelers, pre-replication complex, and helicases.
(B) Heatmap of chromatin modifying complexes.
(C) Heatmap of SMC complexes.

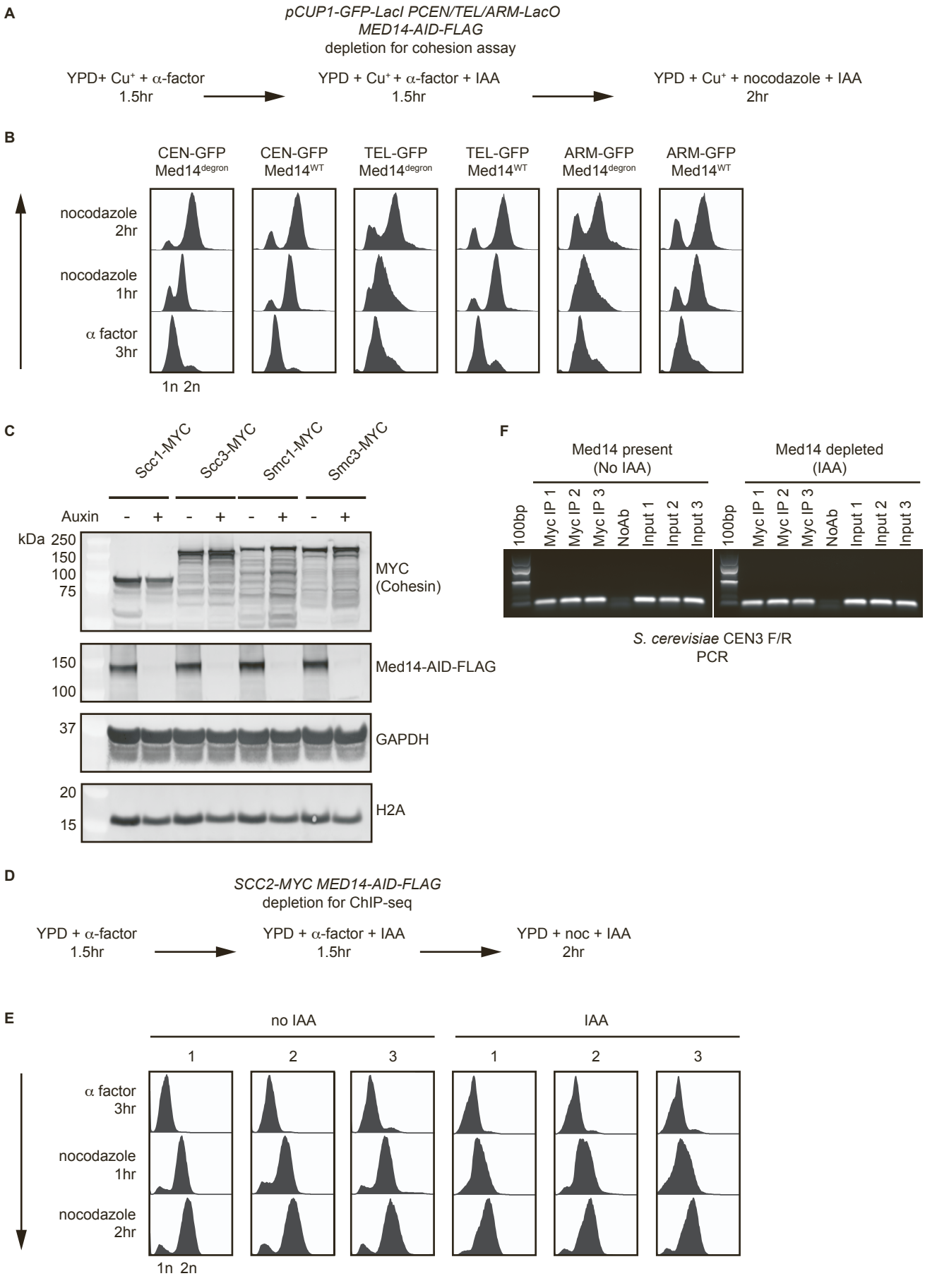
A
pCUP1-GFP-LacI PCEN/TEL/ARM-LacO
MED14-AID-FLAG
depletion for cohesion assay
YPD+ Cu+ + α-factor
1.5hr
YPD + Cu+ + α-factor + IAA
1.5hr
YPD + Cu+ + nocodazole + IAA
2hr
B
CEN-GFP Med14degron
CEN-GFP Med14WT
TEL-GFP Med14degron
TEL-GFP Med14WT
ARM-GFP Med14degron
ARM-GFP Med14WT
nocodazole 2hr
nocodazole 1hr
α factor 3hr
1n 2n
C
Scc1-MYC
Scc3-MYC
Smc1-MYC
Smc3-MYC
Auxin - + - + - + - +
kDa 250
150
100
75
MYC (Cohesin)
150
100
Med14-AID-FLAG
37
GAPDH
20
15
H2A
F
Med14 present (No IAA)
Med14 depleted (IAA)
100bp
Myc IP 1
Myc IP 2
Myc IP 3
NoAb
Input 1
Input 2
Input 3
S. cerevisiae CEN3 F/R PCR
D
SCC2-MYC MED14-AID-FLAG
depletion for ChIP-seq
YPD + α-factor
1.5hr
YPD + α-factor + IAA
1.5hr
YPD + noc + IAA
2hr
E
no IAA
IAA
1
2
3
α factor 3hr
nocodazole 1hr
nocodazole 2hr
1n 2n

Figure S6. Cell synchronization for cohesion assay and ChIP-seq in the absence of Med14, and levels of cohesin after Med14 depletion and ChIP validation by PCR, related to figure 5 and figure 6

(A) Schematic of the experimental procedure for depleting Med14 within one cell cycle for the cohesion assay experiment in Figure 5E.
(B) FACS analysis of the DNA content from one replicate of the cohesion assay experiment in Figure 5E. The nocodazole arrest was not complete for all strains however, only large-budded cells with a single unseparated DNA mass were scored for loss of sister chromatid cohesion.
(C) Depletion of Med14 does not affect levels of cohesin. Cycling yeast cells were grown in the presence of auxin for 4 hours. Whole cell extracts were normalized and levels of Myc tagged subunits of cohesin and Med14 depletion were analyzed by immunoblot. GAPDH and H2A served as loading controls.
(D) Schematic of the experimental procedure for depleting Med14 within one cell cycle for the ChIP-seq experiment
(E) FACS analysis of the DNA content of the cells used for ChIP-seq.
(F) Validation of Scc2-Myc chromatin immunoprecipitations used for ChIP-seq. PCR was used to verify the presence of centromere 3 DNA in the Scc2 chromatin immunoprecipitations and inputs from each replicate compared to a no antibody (NoAb) control. No amplification was observed in the NoAb controls.